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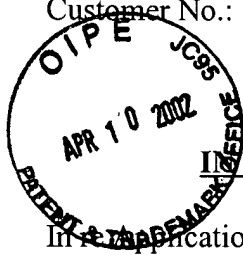
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PATENT TRADEMARK OFFICE

Docket No: 4305/1E144-US1



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hans Henrik Ipsen; Michael Dho Spangfort; and Jorgen Nedergaard Larsen

Serial No.: 09/270,910

Art Unit: 1644

Confirmation No.:

Filed: March 16, 1999

Examiner: P. Huynh

For: **NOVEL RECOMBINANT ALLERGENS**

RECEIVED

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DECLARATION OF DR. T.P. KING UNDER 37 C.F.R. § 1.132

1. I, T.P. King, am a Professor Emeritus at the Rockefeller University. I am head of the Laboratory of Biochemistry. My research is directed to the studying the immunogenicity of allergens, including those derived from Vespid venom, for use as therapeutic vaccines. My work includes elucidating the three-dimensional structure of the allergens. A copy of my Curriculum Vitae accompanies this Declaration (Exhibit A). I assert that I am over 18 years of age and am a citizen of the United States of America.

2. I am not employed by ALK-Abelló/AS, nor am I a co-inventor of the subject matter of the above-identified patent application (hereinafter "subject application"). ALK-Abelló/AS and its U.S. subsidiary, ALKVespa, have in the past and continue to provide me with

research reagents and funding. I am the inventor on several issued patents licensed to ALK-Abelló/AS, a company which I understand owns the rights in the instant patent application. However, I have no rights or interest in the invention described in the subject application. Darby & Darby, patent counsel for ALK-Abelló in the U.S., have retained me as an expert, and they are compensating me for the services I provide.

3. I have read the subject application (including its specification and claims pending as of October 10, 2001), as well as the following correspondence between the Applicants and the Examiner: the Office Action dated February 13, 2001, the Applicants' Response dated July 13, 2001, and the Final Office Action issued on October 10, 2001. The subject application describes novel recombinant allergens, derived from naturally occurring allergens, which contain at least one amino acid substitution at a surface-exposed, conservative (among homologous proteins) residue, which residue is within a region that is an epitope for a B lymphocyte (*i.e.*, is an antibody-binding region). Such recombinant allergen has essentially the same α -carbon backbone tertiary protein structure as the naturally occurring counterpart, but has a reduced binding affinity for specific IgE binding. The pending claims are directed to the recombinant allergen, as well as to pharmaceutical compositions comprising the recombinant allergen for use as a vaccine. The recombinant allergen thus initiates a B lymphocyte-mediated protective immune response, without eliciting, or eliciting a weakened, allergic response.

4. I have been requested to comment on whether the subject application would have provided enough information to allow a person having ordinary skill in the field of the application to generate a recombinant allergen having the described characteristics from any taxonomic order having known homologous family members. I understand from patent counsel that my comments should be based only on the disclosure in the application and on common general knowledge in this field as of a date no later than March 18, 1998, the earliest effective filing date of the application.

5. It is my understanding that the Examiner is of the opinion that the subject

application does not provide sufficient information to have enabled a person skilled in the art to make a recombinant allergen, having the above-identified characteristics, from a taxonomic order other than the two allergens specifically exemplified in the subject application (Bet v I, from order *Fagales*, and Ves v5 from *Vespula*). I understand further that the Examiner has based his opinion on the following suppositions:

- (i) that there is insufficient guidance in the subject application as to the exact amino acids and the specific type of amino acid residues that could be substituted in any allergen and would result in that allergen having reduced IgE binding while retaining the α -carbon backbone tertiary structure;
- (ii) that the state of the art as of the effective filing date of the subject application recognized and acknowledged the unpredictability of the relationship between the primary amino acid sequence of a protein and its tertiary structure as being limited to mutagenesis methods, thus accordingly, the effect of a single amino acid substitution on the ability of the allergen to bind IgE would have been likewise unpredictable (absent additional working examples for every allergen disclosed in the specification); and
- (iii) that absent *in vivo* data regarding the administration of compositions comprising a recombinant allergen made according to the invention, the state of the art was such that use as a vaccine was highly unpredictable regarding the following (a) whether a reduction of IgE binding of 5% to 10% would be effective as a vaccine; (b) and whether the protein would remain activated, reach its designated target area, not elicit unwanted side-effects, and (c) whether any of the disclosed routes of administration and effective doses would be effective.

The Examiner stated in the Final Office Action dated October 10, 2001, at page 5, ultimate paragraph:

For these reasons, the specification as filed fails to enable one skill[ed] in the art to practice the invention without undue amount of experimentation. As such, further research would be required to practice the claimed invention.

6. I respectfully disagree with the Examiner's determination that the application fails to provide sufficient information to enable one of ordinary skill in the field to generate recombinant allergens, having the characteristics described in paragraph 3 above, using the methods described (and exemplified for two very distinct allergens) in the specification of the subject application. Rather, based on my review of several literature publications, discussed below, and my experience in this field, I have concluded that:

- (i) no later than March 18, 1998, various means were available for predicting the specific amino acids that are conserved in homologous proteins, the substitution of which would not alter the α -carbon tertiary structure, given the known relationship between sequence identity and structure for homologous proteins (see Branden, C., et al., *Introduction to Protein Structure*, Garland Publishing Inc. 1999; 348-350: textbook-Exhibit B);
- (ii) no later than March 18, 1998, various means were available for predicting the amino acids and the specific type of amino acid residues that could be substituted in any allergen and would result in that allergen having reduced IgE binding, given the state of the art surrounding surface-localized B cell epitopes and methods of identifying immunodominant residues relevant for determining antibody binding (see Davies et al., Proc. Natl. Acad. Sci. 1996; 93:7-12-Exhibit C; and Van Regenmortel, M.H.V., *Structure of Antigens*, CRC Press Inc 1992:1-27-Exhibit D); and

- (iii) the specification of the subject application contains sufficient information to predict and make modified, recombinant allergens containing mutations in immunodominant B cell epitopes, using methods other than site-directed mutagenesis, and such methods were routinely employed, as discussed further below.

7. As stated in the subject application (page 16, line 36, to page 17, lines 6-11), the method of preparing a recombinant allergen involves first identifying amino acid residues with more than 70% identity in homologous members within the taxonomic order, defining at least one patch of conserved amino acid residues being coherently connected over a region spanning at least 400Å on the three-dimensional surface of the allergen, which has a solvent accessibility of at least 20% and comprises a B cell epitope, and then substituting at least one solvent-accessible amino acid residue in the epitope with a non-conservative residue while preserving the structure of the carbon backbone. As stated in Branden, C., et al., *supra*:

...it is frequently found that two proteins with sequence identify below the level of statistical significance have similar functions and similar three-dimensional structures. In these cases, functionally important residues are identical and usually such residues form sequence patterns or motifs that can be used to identify other proteins that belong to the same functional family...if significant amino acid identity is found with a protein of a known...structure...using computer modeling....this model can serve as an excellent basis for identifying amino acid residues [on another protein] involved in...antigenic epitopes (page 348).

and

Homologous proteins always contain a core region where the general folds of the polypeptide chain are very similar. This core region contains...the...elements that build up the interior of the protein. Even

distantly related proteins with low sequence identity have similar scaffold structures...The greater the sequence identity, the more closely related are the scaffold structures...(page 349).

In addition, Van Regenmortel, *supra* states at page 8 that:

These methods have led to the conclusion that only 3-8 residues of the antigen are critical to antibody binding....This makes it possible to define a so-called "energetic epitope" [energetics of binding]...the additional residues present in the structural epitope can then be viewed as playing a scaffolding role necessary to keep the critical, interacting residues in their proper position.

Further, it is stated in Davies et al., *supra* at page 7 that:

Water molecules have been observed in cavities within the interface and on the periphery, where they often form bridging hydrogen bonds between antibody and antigen...and it has been proposed that the bound waters play a major enthalpic role in the binding...

It is evident from the combined teachings of the above-mentioned references (none of which are specific to allergens) that the specific amino acid residues within an epitope directly involved in antibody binding are few, and could have been readily identifiable according to routine methods of those skilled in the field (including empirical testing, based on general knowledge of a protein's structure). It is also evident from Davies et al. and Figure 1 on page 2 of Van Regenmortel that the residues involved in antibody-binding are surface-localized and water (solvent) accessible (*i.e.*, exposed). In addition, Branden et al. demonstrate that the core region of proteins, which comprises the α -carbon backbone and is *always* (emphasis added) similar in homologous proteins, is conserved. Further, as demonstrated in Van Regenmortel, numerous methods, including but not limited to mutagenesis, for determining antigen-antibody binding and assessing antigenic reactivity were well-known at least seven years prior to the filing of the subject application. Taken together with the disclosure of the subject application, which quantitatively defines criteria for selecting residues for

substitution, including solvent accessibility (page 19, line 32, to page 20, line 2), conservation of known tertiary structure (page 19, lines 15-18), and location on the surface of an allergen (page 20, lines 4-14), there is sufficient guidance in the specification to enable one skilled in the art to identify the amino acid residues involved in epitope binding (typically 5-8), and select one residue to substitute with a non-conservative residue that would result in decreased binding to the antibody while maintaining the backbone structure.

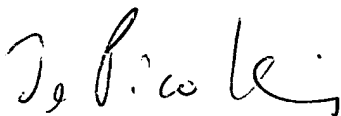
8. As stated and exemplified in the specification, given a known amino acid sequence and tertiary structure of an allergen, *e.g.*, as determined by X-ray and NMR analysis (see specification at page 19, lines 9-11), regardless of the taxonomic order, the surface-localized regions larger than 400Å comprising epitopes could be identified using any methods known in the art, including X-ray crystallography of allergen-antibody complexes, computer modeling, using peptide fragments as cross-reactive antigenic probes, by systematic replacement of selected residues followed by binding assays, using fusion proteins to express potentially antigenic peptides for binding analysis, and by topographic epitope mapping (Van Regenmortel, M.H.V., *supra*). Once determined, patches of highly conserved residues on other homologous proteins within the same taxonomic order (*e.g.*, a common epitope) can be identified by sequence alignment using publicly available databases typically used in the field (see specification at page 24, lines 23-35). The specification particularly exemplifies using a combination of NMR followed by sequence alignment to identify sequences common to homologous proteins (see specification at page 23, lines 31-36). Once common epitopes have been determined, amino acid residues within the regions demonstrating solvent accessibility of at least 20%, which are predicted to be involved in antibody binding, can be identified based on their atomic coordinates within the three-dimensional structure of a homologous taxonomic member (page 24, lines 14-21). Following identification of residues meeting these criteria, recombinant mutants of the allergen can be made with relative ease to one skilled in the art using ordinary recombinant DNA technology. The specification exemplifies cloning the gene of the selected allergen, introducing the mutation by PCR, and expressing the recombinant mutant protein in bacteria (see specification at pages 25-29). Determination of the effect of the mutation on the structure of the α -carbon tertiary backbone can be predicted, if desired by X-ray crystallography

(see specification at page 30, line 22, to page 31, line 4), followed by IgE antibody-antigen binding assays to assess reduced binding affinity (see specification at page 31, lines 17-35). These techniques are not limited to one or two antigens, but may be applied to any known biological antigen.

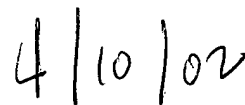
9. Thus, the subject application provided a sufficient basis and methods for making a recombinant allergen by identifying residues within B-cell epitopes in homologous allergens, predicting residues to mutate that would not affect the tertiary structure of the allergen, mutating the residues using ordinary recombinant DNA techniques, and determining the effect on IgG antibody binding. Consequently, such a recombinant allergen that was demonstrated to have reduced binding affinity to IgE would be useful in a pharmaceutical composition administered as a vaccine to elicit an IgG response against the native allergen while reducing the magnitude of the IgE-induced allergic reaction.

10. I declare further that statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Declarant's signature:



Te Piao King, Ph.D.



Date